ELISA: Frequently Asked Questions

What are the differences between BD ELISA products?

We offer matched antibody pairs, BD OptEIA™ ELISA sets, and BD OptEIA ELISA kits.
http://www.bdbiosciences.com/reagents/elisa/index.jsp
Matched antibody pairs include the unlabeled capture antibody, biotinylated detection antibody, and protein standard, and are also sold as individual reagents, if available. A cost-effective approach, these reagents offer a wide range of specificities along with assay design flexibility for researchers developing their own immunoassays for tissue culture supernatant samples.

BD OptEIA sets include reagents sufficient for five or twenty 96-well ELISA plates. Capture antibody, biotinylated detection antibody, streptavidin-horseradish peroxidase, and recombinant standard are included. The components are pre-titered and QC tested as a set, virtually eliminating the need for tedious optimization experiments. Some sets include a fragmented (Fab) detection antibody to reduce background and non-specific binding to auto-antibodies and plasma proteins, such as complement components, acute phase proteins, and fibronectin. A certificate of analysis with lot-specific performance data is included in each set, except for mouse and rat TNF ELISA sets (Cat. No. 558534 and 558535).

BD OptEIA kits include two pre-coated 96-well plates, lyophilized standards, detection antibody, streptavidin-horseradish peroxidase, standard/sample diluent, ELISA diluent, wash concentrate, TMB one-step substrate reagent, stop solution, and plate sealers. The kit contains all the necessary reagents and is developed for superior accuracy, measuring soluble proteins in serum, plasma, and tissue culture supernatants.

What are the differences between the mouse TNF mono/mono ELISA set (Cat. No. 555268) and the mouse TNF ELISA set II (Cat. No. 558534)?

The capture and detection antibody pairs in these sets are different. From our in-house data, we believe that the TNF mono/mono set can detect soluble TNF only. The TNF ELISA set II is believed to be able to detect both soluble TNF and TNF bound to the soluble TNF receptors, but we have not tested this in-house to verify.

How do I store my BD OptEIA kits or sets?

Store kits or sets at 2–8°C. Store the reconstituted standard at –80°C in single-use aliquots for up to six months.

How do I plot a standard curve and determine the curve fitting for BD OptEIA ELISA assays?

Logistic regression is commonly used for ELISA assays. Two common logistic equations are used: four-parameter logistic equation (4PL) and five-parameter logistic equation (5PL). The type of logistic equation that will yield the best fit of the data is dependent on the shape of the curve from an assay. If the curve is symmetrical, a 4PL or 5PL regression will yield similar results. When the curve is asymmetrical, 5PL regression will yield better results.

What type of software is needed to graph a 4-parameter or 5-parameter curve?

You can use SoftMax Pro by Molecular Devices, SigmaPlot® by Systat Software Inc., or others.

ELISA: Frequently Asked Questions

I do not have software that will perform a logistic regression standard curve. What should I use to analyze my data?

You can use a linear regression curve in Microsoft Excel. If you use this type of curve, only use a maximum of 5 points on the curve. However, you cannot directly plot a 4-parameter curve with Microsoft® Excel®.

Which ELISA plates do you recommend?

We recommend using either BD Falcon™ (Cat. No. 353279) or Nunc Maxisorp™ (Cat. No. 446469).
http://www.bdbiosciences.com/external_files/dl/doc/tds/live/web_enabled/tds_LSR00178.pdf
http://www.nuncbrand.com/us/page.aspx?ID=245

Can one coating buffer be used for all ELISAs?

No. We have two different types of coating buffers:

- **Coating Buffer pH 9.5:** 0.1 M sodium carbonate, pH 9.5, 7.13 g $NaHCO_3$, 1.59 g Na_2CO_3; q.s. to 1.0 L; pH to 9.5 with 10 N NaOH.
- **Coating Buffer pH 6.5:** 0.2 M sodium phosphate, pH 6.5, 12.49 g Na_2HPO_4, 15.47 g NaH_2PO_4; q.s. to 1.0 L; pH to 6.5.

Freshly prepare or use within 7 days of preparation, store at 2–8°C.

Note: use pH 6.5 coating for these ELISA sets.

ELISA set	Catalog Number
Human ICAM-1	551424
Mouse IL-10	555252
Mouse IL-12 p40	555165
Mouse IL-12 p70	555256
Mouse MCP-1	555260
Mouse TNF	555268
Rat IL-10	555134

How do I optimize the capture and detector antibody pair concentration?

Our BD OptEIA kits and sets have been optimized for ELISA assays and utilize Fab fragments for the detector antibody. The recommended protocol must be followed to ensure optimal assay performance. For our ELISA pair reagents, you must to determine the optimal signal and lowest background for the ELISA. The capture antibody (1–4 µg/mL) and detection antibody (0.25–2 µg/mL) should be titrated against each other in a preliminary experiment. An appropriate range of serial dilutions for the cytokine standard should be included. A suggested range is generally provided on the technical data sheet (TDS) for ELISA reagents. Generally, use of the capture antibody at 2 µg/mL and the detecting antibody at 1 µg/mL is a good starting point.

What is the best way to prepare recombinant cytokine standards?

For BD OptEIA sets and kits, read the instructions for standard preparation/handling of recombinant cytokine standards carefully. For maximum recovery, the vial of cytokine should be quick-spun before opening. Lyophilized cytokines should be reconstituted as indicated in the TDS.

ELISA: Frequently Asked Questions

Allow the standard to equilibrate for at least 15 minutes before making dilutions. Vortex gently to mix. After reconstitution, **immediately** aliquot standard stock in polypropylene vials at 50 µL per vial and freeze at –80°C for up to 6 months. If necessary, store at 2–8°C for up to 8 hours prior to aliquotting/freezing. Do not leave reconstituted standard at room temperature (RT).

For single-vial recombinant proteins, read the TDS for each recombinant cytokine carefully. Follow the ELISA instructions. For use as an ELISA standard, we recommend a carrier protein concentration of 5–10 mg/mL.

Is the enzyme conjugate in BD OptEIA products avidin-HRP or streptavidin-HRP?

We have been switching from Av-HRP to SAv-HRP, and most of our BD OptEIA kits and sets have Sav-HRP. This switch was because of our effort to replace mercury-containing products in our BD OptEIA products.

When using peroxidase as the enzyme for color development, avoid sodium azide in wash buffers and diluents, since this is an inhibitor of peroxidase activity.

Where can I buy the stop solution (2 M sulfuric acid)?

Sulfuric acid is sold by chemical companies such as Sigma or VWR. The stock solution is 18.0 M and should be diluted to 2.0 M with distilled water.

How can I quickly check the enzyme substrate reagent activity?

To check the activity of the enzyme/substrate system, coat 1 µg/mL of biotinylated detection antibody in several wells in binding buffer for a few hours. After blocking, wash several times, and then proceed with the cytokine ELISA protocol. If the enzyme/substrate system is active, a strong signal should be seen.

What type of samples can I use with BD OptEIA kits and sets?

Our ELISA kits and sets are optimized for serum, plasma, and tissue culture samples.

When measuring cytokines in complex fluids such as serum and plasma, we recommend using our BD OptEIA™ assay diluent (Cat. No. 555213) which includes irrelevant Ig for diluting samples.

Since experimental designs can differ, it is difficult to determine an overall dilution for all samples. If you have a general idea of the concentration of cytokine in your samples, you will want to make dilutions so that your samples fall in the middle range (or linear range) of the standard curve. For example, make 1:2, 1:10, and 1:100 dilutions to determine a dilution range for your samples.

Are there any stopping points during the ELISA assay protocol?

Yes, the plates can be covered and incubated overnight at 4°C either with the coating antibody or after the blocking steps. The coating antibody or blocking solution must be left on the plate during the incubation period. We do not recommend leaving these solutions on the plate for more than 24 hours.

How do you determine assay sensitivity?

Sensitivity, or minimum detectable concentration (MDC), is confirmed by assaying a minimum of 20 replicates of the zero standard in a single assay. The concentration extrapolated from the standard curve of the average ODs for the standard 0 replicates ±2 SD is the MDC. This value represents the lowest

ELISA: Frequently Asked Questions

value read from the standard curve that can be statistically differentiated from zero. The sensitivity should be verified by assaying a 1:2 dilution of the lowest standard.

Troubleshooting Tips

Observation	Probable cause
Low absorbance	Improper antibody preparation
	Improper standard preparation
	Enzyme or substrate reagent failure
	Improper buffer/diluent used
	Reagents were cold when added to wells
	Incorrect incubation time
	Wells dried out
	Excessive wash/aspiration pressure from automated plate-washer
	Plate reader set at incorrect wavelength
High background	Improper antibody preparation
	Incomplete washing/aspiration of wells
	Substrate contamination
Poor precision	Inaccurate pipetting
	Inadequate mixing of reagents
	Samples contain precipitates
	Wells dried out
	Dirty well bottom
Poor standard curve	Improper dilution/handling of standards
	Incomplete washing/aspiration of wells
	Inaccurate pipetting
	Improper diluent used for standard preparation

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